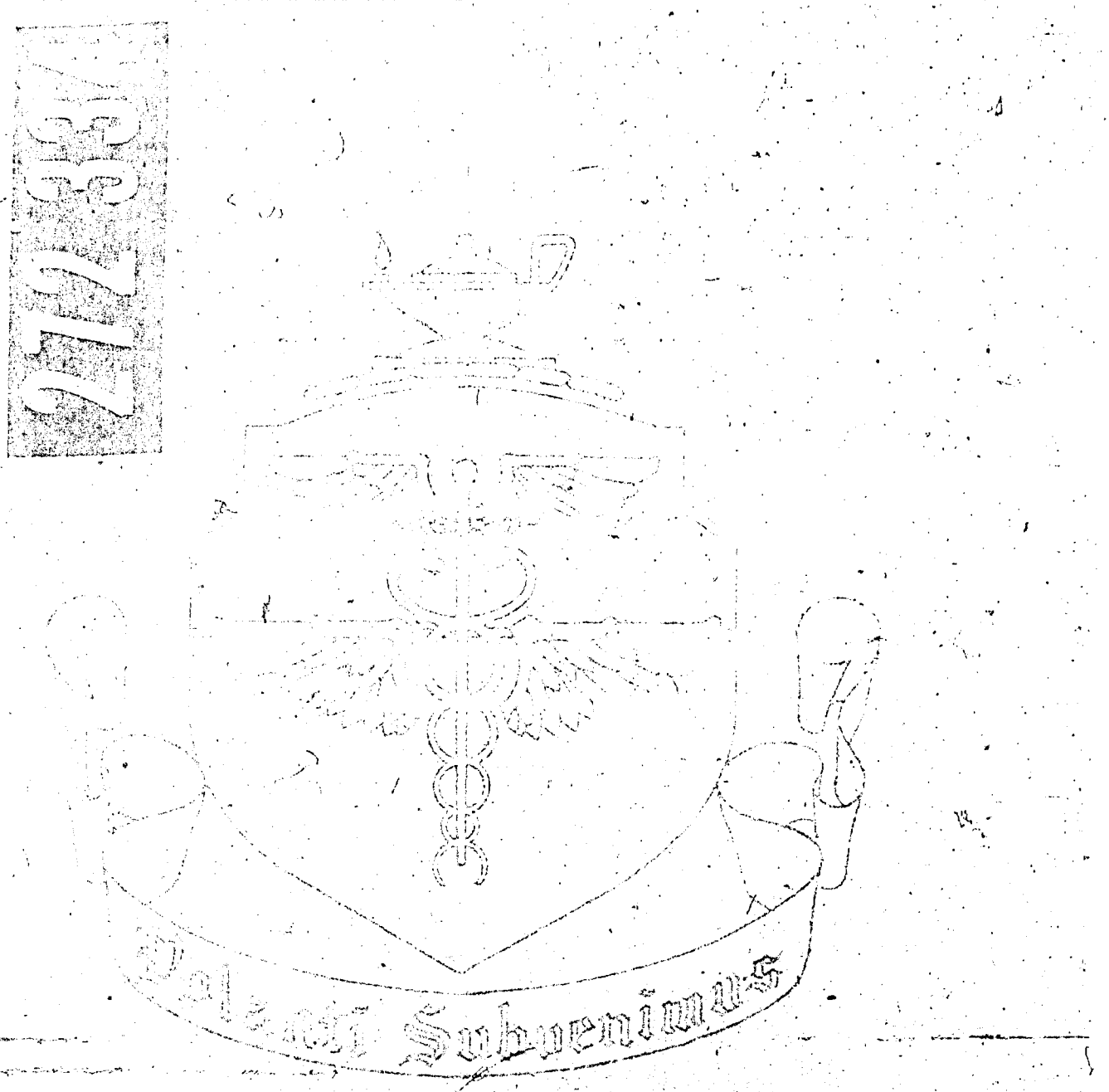

STUDIES ON THE PREVENTION OF CONTAMINATION OF EXTRATERRESTRIAL BODIES

Bacteriologic Examination of Hermetically Sealed Electronic Components

JOSEPH T. CORDARO, M.S.

Microbiology–Cellular Biology Branch

62-18

SCHOOL OF AEROSPACE MEDICINE
USAF AEROSPACE MEDICAL CENTER
BROOKS AIR FORCE BASE, TEXAS

November 1961

7783-59684

STUDIES ON THE PREVENTION OF CONTAMINATION OF EXTRATERRESTRIAL BODIES

Bacteriologic Examination of Hermetically Sealed Electronic Components

Bacteriologic technics to determine the existence of contamination in hermetically sealed electronic components are described. Shelf-stock electronic components were examined as well as those considered typical of being included in the electronic systems of spacecraft. Of the 166 components examined, 11 were contaminated. Paper and mylar-type capacitors were found more likely to be contaminated during fabrication than other types of capacitors examined.

A practical approach for the development of procedures for the sterilization of electronic components is presented.

Biologic decontamination of space vehicles and probes to prevent contamination of celestial bodies was recommended in 1958 by CETEX.[1] Since that time, a number of papers have been published in which the authors expressed in great detail the reasons for sterilizing spacecraft before a lunar or planetary landing is made (2-6). Lederberg and Cowie (2) believed that contamination of a celestial body with earth microorganisms would jeopardize studies on the possible existence of extraterrestrial life. Moreover, as reported by Davies and Communtzis (3), biologic contamination may distort the findings of samplings of prelife organic substances on a celestial body even if no living forms are found.

Although the problem of biologic contamination is of great interest to many biologists, only a few researchers have reported on studies related specifically to the decontamination of spacecraft (3, 4, 5, 7). Davies and Communtzis (3) recommended operational procedures consisting of sterile assembly, built-in disinfection, and terminal sterilization. The use of heat, radiations, and chemicals was recommended for terminal sterilization. Phillips and Hoffman (4) and Wynne (5) presented data to show that hermetically sealed units or components, which are the smallest units of spacecraft, are contaminated with microorganisms during fabrication.

Later, Nowitzky (7) reported on a gas transfer system designed for the surface sterilization of the nose cone and spacecraft. Practical methods for sterilizing electronic components, however, have not been reported.

Hence, the purpose of this report is to present the technics used in the bacteriologic examination of hermetically sealed electronic components with the possibility of developing methods for examining electronic components after sterilization. The results obtained from examining "shelf-stock"[2] components together with a series of components obtained from the McDonnell Aircraft Corporation (MAC)[3] dur-

Received for publication on 26 July 1961

[1] The International Committee on Contamination by International Exploration

[2] Obtained from the Biochemistry Research Laboratory, School of Aerospace Medicine

[3] Obtained November 1960 through the courtesy of Dr. George S. Herman, Medical Director, McDonnell Aircraft Corp., St. Louis, Mo.

ing their work on Project Surveyor are presented. MAC submitted the following information: "While these components were not intended for use in Surveyor, they are considered typical of components which would be included in the electronic systems of that vehicle. Components were selected from various electronic systems used in other projects at MAC. Some of the components were unused, while others had been in various electronic-system tests. None of the components had been used for prolonged periods of time. There had been no attempt to sterilize the components either by MAC or by the component manufacturer."

METHODS AND PROCEDURES

Sterilization of testing chamber and contents

The disassembling and culturing procedures of the electronic components were performed inside a germ-free, flexible, film isolator (fig. 1). The interior and contents of the isolator were sterilized with Cryoxcide (ethylene oxide — 11 percent (66 gm. per can) and fluorinated hydrocarbons—89 percent). The entire contents of two 21-ounce cans of Cryoxcide were poured quickly into large beakers, inside the isolator, to allow the gas mixture to evaporate as soon as possible. As the isolator has a volume of approximately 600 liters, the resulting concentration of ethylene oxide gas was 220 mg. liter. This concentration of ethylene oxide was sufficient to destroy the maximum number ($3 \cdot 10^6$) of bacterial spores (*Clostridium sporogenes* spores) which were dried on metal strips and exposed inside the isolator for 8 hours. After sterilization, the disinfectant gas was displaced by flushing the isolator with filtered, sterile air overnight. To prevent the influx of nonsterile air, in the event that a leak developed during the test, positive pressure was maintained (approximately $\frac{1}{4}$ in. h water column) by continuously pumping filtered, sterile air into the isolator.

Before and during each test run, control tests were performed to check the sterility of the interior of the isolator. Nutrient agar plates, with tops removed, were exposed inside the isolator during the entire operating time. Swabbings of the interior surfaces of the isolator and surfaces of the tools used were made at various intervals, the swabs being placed in thioglycollate broth. Metal strips, containing known concentrations of spores, were exposed during the sterilization procedure. The nutrient agar plates, thioglycollate broth containing the swabs, and the metal strips were incubated at 35° C. for 6 days.

Disassembling procedures

As pointed out by Wynne (5), differences in the construction and composition of the various types of electronic components require the use of different disassembling techniques. Each component was disassembled with the use of hand tools such as pliers, forceps, mortar and pestle, hack saws, etc., so that all interior surfaces of the component were exposed for culturing purposes. Methods were developed, as described below, by performing initial tests on a series of shelf-stock components. Both miniature and standard-size components were included in this study.

1. *Resistors.* Three types of resistors were examined: molded carbon, wire-wound, and porcelain-deposited carbon. The resistors were easily broken up with the mortar and pestle because of their composition.

2. *Capacitors.* The following types of capacitors were examined:

a. Paper and mylar

These capacitors had basically the same construction—i.e., thin layers of paper or mylar and aluminum foil rolled tightly together. They differed, however, in the type of outer covering which was either molded plastic or metal. The molded plastic types were first crushed with the mortar and pestle to expose the contents. Metal-encased types required the use of the hack saw and cutting pliers to remove the contents. The rolled layers of paper or mylar and aluminum foil were carefully separated to expose all possible surfaces which might be contaminated during fabrication.

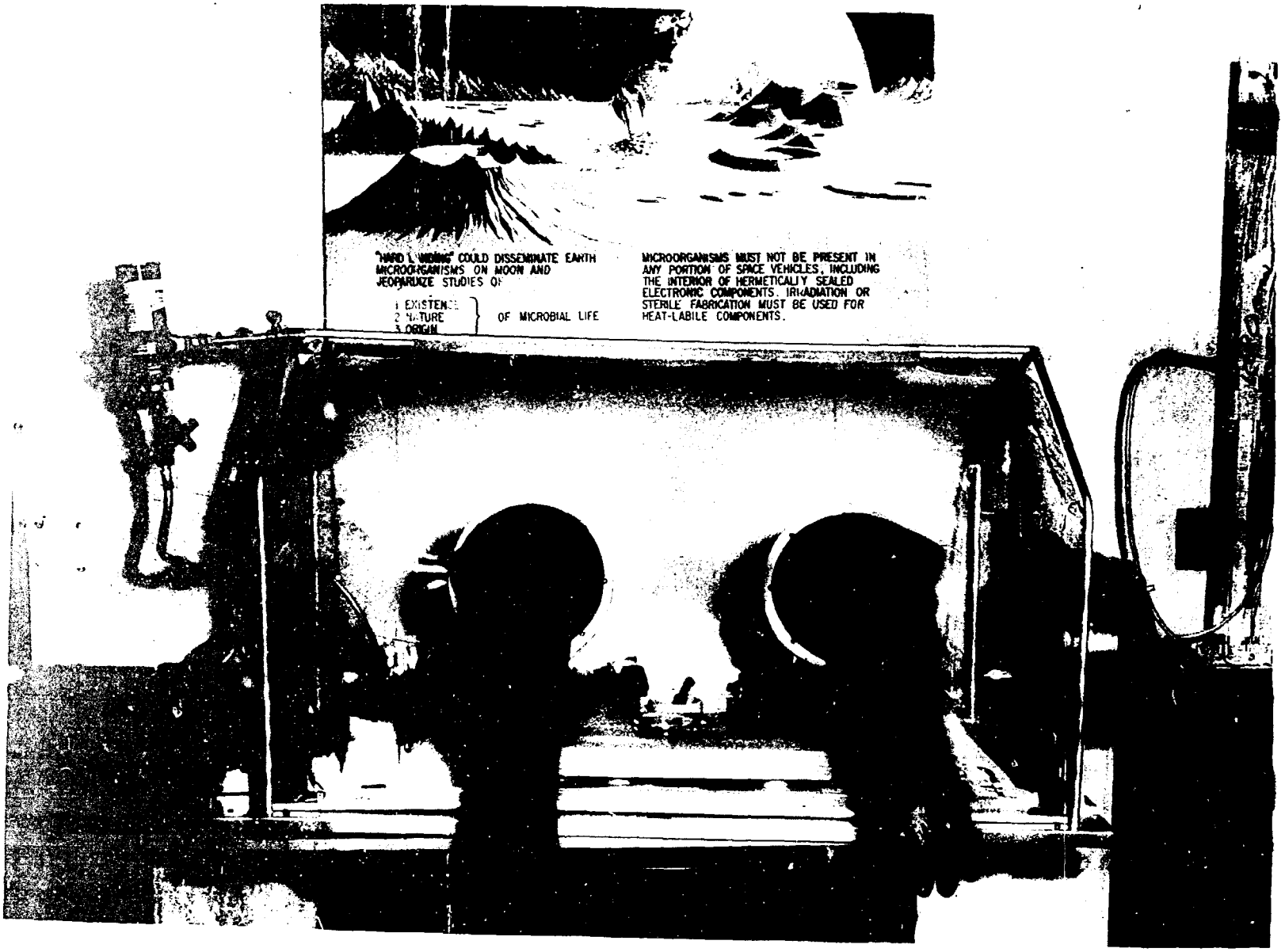

FIGURE 1

Flexible film germ-free isolator.

b. Ceramic flat disc

This type of capacitor was easily crushed and pulverized with mortar and pestle.

c. Electrolytic

These electrolytic capacitors were miniature, metal-encased, tantalum capacitors which were cut open with the hack saw and cutting pliers. The rolled tantalum capacitors consisted of thin layers of paper impregnated with dielectric paste and thin strips of tantalum metal rolled together and then separated to expose all surfaces. The solid tantalum capacitors were crushed with mortar and pestle.

d. Molded mica

These consisted of several sheets of mica molded in Bakelite, crushed with mortar and pestle.

3. *Diodes.* The diodes examined were miniature, glass-encased types, broken up with the mortar and pestle.

4. *Electronic tubes.* General-purpose, glass-encased tubes were used. These were prepared for culturing by breaking them up with the mortar and pestle and separating the internal parts with pliers and forceps.

5. *Relays, transformers, and potentiometers.* Since these were all metal encased, they were cut open with the hack saw, usually at the top end which contained the connecting poles or wire leads, to expose and remove the contents. Each screw, nut and bolt, or metal plate, holding or binding other parts, was removed. Plastic coverings, such as tape around wire coils or insulation around wire leads, were removed and either separated or cut into small pieces. Wire coils were cut with the hack saw or jeweler's saw across the wire windings and, by careful teasing, as many as possible of the wire surfaces were exposed.

Culturing procedures

The culturing procedure was essentially the same as described by Phillips and Hoffman (4) and Wynne (5). The entire disassembled contents of each component were cultured in thioglycollate broth in wide-mouth, screw-capped bottles and incubated at 35° C. for 6 days. Observations for cloudiness, indicating possible bacterial growth, were made daily. A similar intact component was also placed in thioglycollate broth to check sterilization of the outer surface of the component with Cryoxcide. When only one component was available for examination, the intact component was immersed in the broth and shaken thoroughly. It was then removed with sterile forceps and disassembled. If the component was too large to be immersed in the broth bottles, a moistened cotton swab was streaked over the entire surface of the component and placed in a tube of thioglycollate broth. Both the rinse-broth and the tubes containing the swabs were incubated as described above.

Broth cultures showing cloudiness within the 6-day incubation period were checked microscopically for microorganisms. Positive cultures were streaked to nutrient agar plates for aerobic growth and inoculated into thioglycollate broth for anaerobic growth. At the end of the 6-day incubation period, all negative cultures (not cloudy) were treated as follows: Five ml. of the broth were mixed thoroughly and removed with a sterile pipet. At the same time, 1 ml. of this broth was inoculated into a tube containing 5 ml. of thioglycollate broth to dilute any possible bacteriostatic agent present in the original broth containing the disassembled component. One drop was placed on a sector of a nutrient agar plate, and the remaining broth (approximately 4 ml.) was placed in a sterile tube. Then the broth that was placed in the sterile tubes was inoculated with *Pseudomonas aeruginosa* to show the ability of the broth to support growth. Nutrient agar plates and thioglycollate broth tubes were incubated at 35° C. for 48 hours. The original broth bottles containing the disassembled components were incubated for an additional 2 weeks at room temperature. Positive cultures developing at the end of the 2 weeks were treated as described above. Cultures of contaminated components were stored in the refrigerator for future identification.

RESULTS

The results of the bacteriologic examination of the hermetically sealed, electronic components are shown in table I. Of a total of 166 components tested, 11 were contaminated internally. This table shows that of 101 capacitors examined, 9 were contaminated. Furthermore, 1 of 4 transformers and the 1 magnetic modulator were contaminated. The other types of electronic components tested yielded negative results. All but 2 of the contaminating microorganisms which were isolated were spore-forming bacteria. In one instance, anaerobic streptococci were isolated from a molded mica capacitor. The magnetic modulator yielded a gram-negative organism. Table II shows the results of the specific types of capacitors tested. These results indicate that paper and mylar capacitors are more likely to be contaminated during fabrication than any of the other types of capacitors. Table III shows the results of the examinations of the components obtained from MAC.

DISCUSSION

The results obtained in this study confirm the existence of contamination of the interior of hermetically sealed electronic components as previously reported by Phillips and Hoffman and Wynne. Admittedly, the number of electronic components examined was small. A sufficient number of components were examined, however, to serve as a basis for developing procedures for the microbiologic examination of electronic components after sterilization.

Studies on the prevention of contamination of extraterrestrial bodies with earth microorganisms which may be trapped during fabrication on the interior surfaces of hermetically sealed electronic components involve several important factors. First, the existence and rate of contamination of the various types of components should be determined. A knowledge of the composition and assembly of the electronic components would be of value in the disassembling procedures in order to expose all interior surfaces for culturing purposes. Second, for those components that are not sensitive to heat or radiation, procedures for

TABLE I

Internal contamination of electronic components

Type of component	Number examined	Number positive
Capacitors	101	9
Resistors	45	0
Diodes	5	0
Electronic tubes	5	0
Relays	2	0
Transformers	4	1
Magnetic modulator	1	1
Micropositioner	1	0
Potentiometers	2	0
Total	166	11

TABLE II

Internal contamination of different types of capacitors

Capacitor	Number examined	Number positive
Paper	31	5
Mylar	16	3
Electrolytic	14	0
Ceramic flat disc	25	0
Molded mica	25	1
Total	101	9

TABLE III

Internal contamination of components obtained from McDonnell Aircraft Corporation

Type of component	Number examined	Number positive
Capacitors		
Mylar	4	2
Paper	6	1
Ceramic flat disc	6	0
Electrolytic	4	0
Resistors		
Molded carbon	2	0
Wire-wound	10	0
Porcelain deposited-carbon	18	0
Diodes	5	0
Electronic tubes	5	0
Relays	2	0
Transformers	4	1
Magnetic modulators	1	1
Micropositioner	1	0
Potentiometers	2	0
Total	70	5

sterilizing them compatible with the operational characteristics of the components should be developed. Either sterile assembly or built-in disinfection technics would be required for components that are sensitive to heat and radiation. Finally, routine procedures for examining representative numbers of electronic components after sterilization and assembly should be developed. Regardless of the method employed, there is no definite assurance that all the components will be sterile. The examination of representative numbers of the different types of electronic components would at the most give only a relative assurance that components are sterile when assembled or treated in a similar manner. Selection of the representative number of components to be examined would depend on qualitative and quantitative studies of the extent of contamination of electronic components.

Preliminary studies on the effects of heat and radiation on electronic components that have been deliberately contaminated with known concentrations of bacterial spores during fabrication will be conducted at the School of Aerospace Medicine for the purpose of developing procedures to sterilize electronic components. By this procedure the time-temperature exposures and the radiation dosage requirements for the sterilization of electronic components can be determined. This study cannot be carried out effectively if only natural contamination is used, because natural contamination occurs infrequently and is presumably of low level. Phillips and Hoffman (4) reported 21 of 123 components contaminated; whereas, in the studies made in the Microbiology-Cellular Biology Branch at the School of Aerospace Medicine, 11 of 166 components were contaminated.

REFERENCES

1. Development of international efforts to avoid contamination of extraterrestrial bodies. Science 128:887-889 (1958).

2. Lederberg, J., and D. B. Cowie. Moondust. Science 127:1473-1475 (1958).

3. Davies, R. W., and M. G. Communitis. The sterilization of space vehicles to prevent extraterrestrial biological contamination. *In* Tenth International Astronautics Congress. Jet Propulsion Lab. External Publ. No. 698. Pasadena, Calif., 1959.

4. Phillips, C. R., and R. K. Hoffman. Sterilization of interplanetary vehicles. Science 132:1001-1005 (1960).

5. Wynne, F. S. Sterilization of space vehicles: The problem of mutual contamination. *In* Lectures in Aerospace Medicine, School of Aerospace Medicine, USAF Aerospace Medical Center (ATC), Brooks AFB, Tex., 1961.

6. De Pinna, H. H. Spacecraft sterilization: Its influence on spacecraft instrumentation design and requirements. Presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1961.

7. Nowitzky, A. M. Spacecraft sterilization: The grand requirement. Lockheed Missiles and Space Division, Van Nuys, Calif., 1961.

8. Creel, H. F. Personal communication. McDonnell Aircraft Corporation, St. Louis, Mo., 1961.